## REMARKS

### The Office Action

Claims 1-18 are pending. Claims 15-17 are withdrawn as being drawn to nonelected subject matter. Claims 1-14 and 18 stand rejected for lack of enablement, and for obviousness in view of Friedrich et al. (Genes Devel. 5:1513, 1991) in combination with one or more of St-Onge et al. (Nucleic Acids Res. 24:3875, 1996), Zhang et al. (Biochem. Biophys. Res. Comm. 227:707, 1996), and Bremer (Nucleic Acids Res. 20:5484, 1992). Claims 5-7 stand further rejected for indefiniteness. Each of these matters is discussed below.

### The Invention

In general, the invention as presently claimed features a mouse having two transgenes. The first transgene, which includes a regulatory gene encoding a regulatory protein, is integrated into an endogenous gene such that two events occur. First, the endogenous gene is mutated by the insertion of the transgene. Second, expression of the regulatory gene is regulated by the promoter of the endogenous gene.

The second transgene includes a gene operably linked to a regulatory sequence that is under the control of the regulatory protein encoded by the first transgene; this gene is referred to hereinafter as "the regulated gene." Gene regulation may be positive (i.e., the expression of the regulated gene may be increased in the presence of the regulatory protein) or negative (i.e., the expression of the regulated gene may be decreased in the presence of the regulatory protein). In either case, because the expression pattern of the

regulatory protein is determined by expression of the endogenous gene, the end result is that it is the endogenous gene's expression that determines how the regulated gene is expressed.

One feature of the claimed mice is that the regulated gene can encode any of a number of proteins. For example, the regulated gene may encode a protein such as green fluorescent protein, luciferase, or chloramphenicol acetyltransferase that allow for detection of cells expressing the regulated gene. Alternatively, the regulated gene may encode a cell ablation factor, such as those described at page 19 of the specification. Expression of the cell ablation factor results in the death of cells in which the factor is expressed. In another variation, described on pages 22 and 23 of the specification, the regulated gene is a gene of interest so that the effect of expression of that gene in a pattern determined by the promoter of the endogenous gene can be ascertained. In still another variation, described on pages 23 and 24 of the specification, the regulated gene may be a neoplastic factor such as an oncogene, allowing for the generation of mice that are prone to develop tumors in the cells in which the oncogene is expressed.

As is clear from Applicant's specification, these mice may be readily generated using the so-called "binary mouse system." In this system, a mouse that contains the first transgene is mated with a mouse that contains the second transgene, thus producing the mouse of claim 1. One advantage of the binary mouse system is that various lines of mice can be produced through mating rather than through genetic manipulations. For example, if a first transgene integrates such that the promoter of a mutated endogenous gene is driving expression of the regulatory gene in a cell type-specific manner, that

mouse can be mated with a second mouse containing a transgene encoding GFP to produce a reporter mouse, or it can be mated with a second mouse containing a transgene encoding a cell ablation factor. Indeed, any protein encoded by a regulated gene can be placed under control of the regulatory protein simply by mating mice. Using prior art methods, if one wished to substitute one protein for another (e.g., a cell ablation factor for GFP), one would need to produce a new construct containing the gene encoding the second protein and then, through the use of random viral integration or targeted homologous recombination, produce a mouse in which the second protein is regulated by the same promoter that was regulating expression of the first protein. Clearly, the binary mouse system provides a tremendous advantage over prior art methods by enabling such production of panels of mice more quickly, more simply, and less expensively than conventional techniques.

These mice, the production and use of which are fully enabled by the present specification, are nowhere taught or suggested by the prior art, as is indicated by Applicant's responses to the current rejections presented below.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-14 and 18 stand rejected for lack of enablement based on the Office's assertion that it would require undue experimentation to use the claimed mice. Applicant respectfully traverses this rejection.

The claims as amended are directed to a mouse having two transgenes. The first transgene is integrated into an endogenous gene of the mouse. The transgene itself

includes a regulatory gene encoding a regulatory protein, and is integrated into the endogenous gene in such a way that the endogenous gene is mutated and the regulatory gene is positioned for expression under control of the promoter of the endogenous gene. The second transgene includes a gene operably linked to a regulatory sequence regulated by the regulatory protein encoded by the first transgene. The claims further require that the regulatory protein modulates expression of the gene operably linked to the regulatory sequence.

The issue raised by the Office in the first Office action in this case is that the claims encompass mice having no phenotype. The Office states that the specification "fails to teach how to use the mice with the claimed genotype but without any phenotype. Therefore, one skilled in the art would not know how to use the invention." Applicant replied that the specification did teach how to use the claimed mice, regardless of whether there is a readily identifiable phenotype, in methods such as the conditional ablation of cell lineages expressing mutant genes, spatiotemporal phenotypic analysis of disrupted genes, and conditional expression of genes of interest.

In the present Office action, the Office has maintained the rejection of claims 1-14 and 18 on the basis that "the specification does not teach that the mice have any readily identifiable phenotype." This concern is unwarranted. As discussed above, the claims define what Applicant states is the invention: any mouse having the two transgenes recited in the claims. A phenotype is neither required by the claims, nor would it be appropriate to require a particular phenotype, because the phenotype will depend in each

case on which endogenous gene is subject to the integration event and which second transgene is employed.

Moreover, Applicant points out that the lack of a described phenotype is not relevant to the enablement of the claims. For a claim to be enabled, one skilled in the art must be able to make and use the claimed invention to its full scope without undue experimentation. The Office is in agreement that one skilled in the art of mouse transgenics could make the claimed mice. As for the use of these mice, Applicant reiterates that mice without phenotypes can be used for all the reasons set forth above and in the previous reply. For example, a mouse having a mutation in an endogenous gene but no resulting phenotype is of interest precisely because of its lack of phenotype. This mouse may be used, for example, to study the expression pattern of the mutated endogenous gene by using, as the regulated gene, a gene encoding a reporter such as GFP. In yet another example, in which the ability of a gene to "complement" a mutated gene is examined, a mouse may have a mutation in an endogenous gene (resulting from the integration of the first transgene), but, if the regulated gene complements the mutated endogenous gene, would not have a phenotype. Again, the lack of a phenotype is of interest; in this case, the lack of phenotype demonstrates that the regulated gene can complement the endogenous gene. Each of these examples demonstrates Applicant's point that a phenotype is not, and should not, be required by the present claims, or for enabling the use of the presently claimed mice.

Withdrawal of this basis of the rejection of the claims for lack of enablement is respectfully requested.

The Office also bases its enablement rejection on the concern that a lack of phenotype may occur because the regulatory protein is not expressed at a level sufficient to modulate expression from the second transgene. Applicant has addressed this concern by amending claim 1 to require that the regulatory protein modulates expression of the gene operably linked to the regulatory sequence.

The rejections under 35 U.S.C. § 112, first paragraph, may be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 5-7 are further rejected because of the reference in claim 5 to "a second transgene." This rejection is moot in view of the amendment of claim 1 and the cancellation of claim 5, and may now be withdrawn.

Rejections under 35 U.S.C. § 103(a)

Claims 1-14 and 18 are yet further rejected as being obvious over Friedrich et al. in view of one or more of St-Onge, Zhang, and Bremer. Applicant respectfully traverses this rejection as it applies to amended claim 1, which is similar to examined claim 14.

Claim 1 now recites a mouse having two integrated transgenes. The first transgene includes a regulatory gene encoding a regulatory protein. This transgene is integrated into an endogenous gene of the mouse such that (i) the regulatory gene is positioned for expression under control of the endogenous gene's promoter, and (ii) the endogenous gene is mutated. The second transgene includes a gene operably linked to regulatory sequence regulated by the regulatory protein.

Friedrich, the primary reference, discloses the use of a promoter trap, in which "expression of a reporter gene can initiate only from an endogenous promoter because the reporter gene lacks its own promoter." As is clear from this and other passages of Friedrich, the authors' intent was to develop a more rapid screen for mutagenized animals. For example, Friedrich summarizes the prior methods as being "laborious and time consuming," and states that "a method that would allow screening and selection for mutations in vitro would be useful." To this end, Friedrich developed a screen that "involves the introduction of a reporter gene preceded by a splice acceptor into ES cells." Friedrich further characterizes the ideal reporter gene as being one that "should be innocuous, allow selection for mutagenic insertions, and include a means to easily monitor the tagged promoter once the ES cell clone has been used to create chimeras and transgenic lines."

Friedrich therefore is solely focused on a singular problem, namely the development of a method for more easily selecting insertion mutations in mice. Friedrich teaches only the use of reporter genes, and is silent on the use of a regulatory gene, as is required in claim 1.

St-Onge, the second reference relied upon by the Office, uses mice that contain three transgenes. The first transgene includes the TetR/VP16 transactivator gene under the control of the human cytomegalovirus early gene 1 promoter-enhancer. The second transgene contains the CMV promoter and a nuclear *β-galactosidase* gene separated by an SV40 polyadenylation signal flanked by two *loxP* sites. The third transgene contains a gene encoding Cre recombinase under the control of a basal CMV promoter fused to

seven copies of the tetO sequences. In these mice, expression of the Cre gene is initially prevented by the administration of tetracycline. When tetracycline is withdrawn, the Cre gene is expressed, resulting in the site-specific recombination between the two loxP sites and consequently expression of the *β-galactosidase* gene.

There are at least two important distinctions between the mice of St-Onge and the claimed mouse. First, all three of St-Onge's transgenes are intended to be ubiquitously expressed (notwithstanding some "variability and mosaicism associated with the CMV promoter"). In contrast, in the mouse of the present invention, the first transgene may be expressed in a spatially restricted manner, depending on the expression pattern of the gene into which the transgene inserts. Moreover, St. Onge's transgene does not necessarily mutagenize an endogenous gene, while mutagenization of an endogenous gene is a requirement in the claimed mouse. The Office nonetheless contends that one skilled in the art would have been motivated to combine the teachings of Friedrich and St-Onge "to provide temporal control of another gene" in a transgenic mouse. The Office has provided no support for this conclusion. As the Federal Circuit recently observed:

> Most if not all inventions arise from a combination of old elements. . . . Thus, every element of a claimed invention may often be found in the prior art. . . . However, identification in the prior art of each individual part claimed is insufficient to defeat patentability of the whole claimed invention. . . . Rather, to establish obviousness based on a combination of the elements disclosed in the prior art, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that was made by the applicant.

*In re Kotzab*, 217 F.3d 1365, 1369-70, 55 USPQ2d 1313, 1316 (Fed. Cir. 2000) (citations omitted). The Office can satisfy the burden of showing obviousness of the combination "only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references." *In re Fritch*, 972 F.2d 1260, 1265, 23 USPQ2d 1780, 1783 (Fed. Cir. 1992).

As discussed above, the prior art cited by the Office provides no motivation to produce the mouse of claim 1. Friedrich was solely focused on the use of reporter genes in promoter trap-mediated mutagenesis as a means by which the efficiency of this method could be improved, and does not consider the use of any other gene or combination with a second transgene construct. St-Onge uses a ubiquitously expressed regulatory protein encoded by a non-mutagenizing transgene to control Cre recombinase expression and is entirely silent on the idea of using promoter trap constructs. Neither reference provides any objective teaching that would lead an individual to combine them in such a manner so as to result in the claimed invention. Indeed, combining the references as proposed by the Office would further the goal of neither Friedrich nor St-Onge.

In view of the foregoing, Applicant respectfully requests that the rejection of claims 1-8 and 14 as being obvious over Friedrich in view of St-Onge be withdrawn.

Claims 9-13 are rejected as being obvious over Friedrich et al. in view of St-Onge and in further view of Zhang (claims 9-12) or Bremer (claim 13). Friedrich and St-Onge are discussed above. Zhang discloses the use of green fluorescent protein as a reporter protein. And Bremer simply discloses the VDE restriction site and VDE-mediated

digestion. Neither of these references remedies the deficiencies of Friedrich or St-Onge references by providing the motivation to make the claimed mouse. Reconsideration and withdrawal is respectfully requested.

New claims 20-24

Applicant has added new claims 20-24.

New claim 20 is drawn to the use of a tetracycline repressor fused to a VP16 transcriptional activator for conditional expression of the regulated gene (described, e.g., on page 14 of the specification). According to claim 20, the first transgene encodes a tetracycline repressor fused to VP16, and the second transgene includes a regulated gene operably linked to a regulatory sequence that is modulated by the regulatory protein in the presence of tetracycline, but not in its absence.

New claims 21-24 are drawn to mice in which the regulated gene (i.e., the gene operably linked to the regulatory sequence regulated by the regulatory protein) encodes a reporter protein (claim 21), a cell ablation factor (claim 22), an oncogene (claim 23) or the mutated endogenous gene (claim 24). The only protein whose expression is regulated by a regulatory protein is St-Onge's Cre recombinase. Neither reference even hints at regulating the expression of a reporter gene, cell ablation factor, oncogene, or the mutated endogenous gene by operably linking DNA encoding any of these proteins to a regulatory sequence regulated by a regulatory protein. Accordingly, Applicant submits that these claims are patentable over the cited art.

## CONCLUSION

Applicant submits that the claims are now in condition for allowance, and such action is respectfully requested. If the Office deems that there are remaining issues, Applicants respectfully request a telephonic interview between the Office and the undersigned.

If there are any charges, or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

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